In twelve animals of this 350–450 gm. group the mean red marrow volume was 6.249 ml. ($s = 0.446$ ml.), representing 89 per cent of the total marrow or approximately one-third of the liver, the mean weight of which was 20 gm.

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A Method of destroying a Malignant Rat Tumour *in vivo*

A HYPOTHESIS of tumour formation based on the catalase-hydrogen peroxide mechanism in living cells was published recently¹ following observations on abnormalities occurring in aerobic and anaerobic bacteria due to certain respiratory irregularities². If this hypothesis be correct, then a logical assumption is that agents which inactivate catalase or produce hydrogen peroxide (or free radicals) should have a detrimental effect on tumours.

The treatment of experimental tumours with the catalase inhibitor sodium azide is effective³. Ionizing radiations have long been known to destroy malignant cells, and this may be explained as being due to the production in the presence of oxygen, in fluid systems, *in vivo*, of hydrogen peroxide, the free powerful oxidizing radicals, HO₂, HO, and atomic oxygen⁴ as well as the destruction of the enzyme catalase⁵.

Hydrogen peroxide has been given by daily intraperitoneal injections in animals carrying tumours, without effect⁶. Makino and Tanaka⁷, however, observed temporary retardation of ascites sarcomas in rats on intraperitoneal injection of 1 ml. of 2 per cent hydrogen peroxide and Hollcroft and Lorenz⁸ noticed some improvement of lymphoid tumours in mice after the intravenous injection of hydrogen peroxide. Worrall⁹ mentions the use of peroxide-forming compounds in animals and humans in an attempt to set up catastrophic reactions in cancer cells.

It seemed to me that the only effective way to destroy the malignant cells which are already deficient in catalase¹⁰ and sensitive to over-oxidation is to keep up a continued administration of an active oxidizing agent. Since hydrogen peroxide is an excellent ionizing solvent, and since it is formed and is obviously of great fundamental importance in most living cells, this agent appears to be the one of choice.

Rats implanted with the Walker 256 adenocarcinoma were treated by simply replacing their drinking water with dilute solutions of commercial hydrogen peroxide (Laporte Chemicals, Ltd., London), and maintaining them on a normal diet. The optimal concentration has been found to be 0.45 per cent by weight and with the rats being housed in metal cages (ten animals per cage) the rate of cure is on the average 50–60 per cent. The time taken for complete disappearance of the tumour is usually 15–60 days. This, of course, depends on the size of the tumour when treatment is started. So far, 72 rats have been cured; they are now back on tap water and normal

diet, and will be maintained in this manner for the rest of their natural lives. Ten of these rats have been cured for more than two months; their condition is excellent and there is no sign of recurrence of the tumour.

This treatment has recently been used in four humans with very advanced inoperable tumours. In two of the cases there has been marked clinical improvement with decrease in size of the liver (which contained metastases) and progressive diminution in the blood serum polysaccharide which Keyser¹¹ has shown to be an indication of effective therapy in neoplastic states.

A detailed account of these experiments will be published shortly.

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A Simple Method for staining Mitochondria

EXCELLENT methods for staining mitochondria have been available for many years, but nearly all demand experience and skill. In the course of a general study of the use of osmium tetroxide in the fixation and staining of tissues, which will be published in full elsewhere¹, I have had occasion to re-investigate the use of gallic acid derivatives in the visualization of osmium^{2,3}. Treatment with tannic acid or pyrogallol after osmium tetroxide fixation provides a useful method for staining droplets of neutral fat; but the osmium taken up by the mitochondria, the nuclear membrane, nucleolus, ergastoplasm, etc., is less readily revealed. I have found that it can be made visible by prolonged treatment with alkyl esters of gallic acid, notably ethyl gallate.

Small pieces of tissue are fixed at 0°–4° C. for several hours in 1 per cent osmium tetroxide buffered at pH 7.25 with veronal acetate buffer in isotonic sodium chloride^{4,5}. They are then transferred to a saturated solution of ethyl gallate in 0.25 per cent cresol for twenty-four hours or longer. The tissue now resembles a small piece of coal. It may be embedded in 25 per cent gelatin and cut frozen, or embedded in paraffin (through cedar wood oil) and the sections mounted in Farrants's medium containing ethyl gallate, or in cedar wood oil containing nonyl gallate. But the simplest procedure is to embed in polyethylene glycol wax of suitable hardness, to cut at 0.5–2 μ , float on water and mount the sections individually in glycerine jelly to which ethyl gallate has been added.

As a method for mitochondria this is extremely simple, gives invariably reproducible results, and requires neither skill nor experience. Fig. 1 shows